

### **REMARKS**

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

With respect to Applicants' claim for foreign priority, Applicants note that the Examiner failed to acknowledge Applicants' claim for foreign priority or acknowledge that all certified priority documents have been received. Applicants respectfully request such acknowledgment in the next Official Action.

The Examiner has also noted the application does not contain an Abstract of the Disclosure which Applicants believe is erroneous. An Abstract representing page 35 of the specification was submitted with the original application papers. For the Examiner's convenience, Applicants have resubmitted a copy of the previously filed Abstract.

Claims 2, 3, 5, 7, 8 and 11-13 have been cancelled without prejudice, and claims 1, 4, 6, 9 and 10 have been amended. The claim amendments have been amended to more particularly define the present invention and to put the claims in better form. Support for the claim amendments is readily apparent from the teachings of the specification and the original claims.

Applicants wish to note that unless specifically noted in the arguments below, all of the claim amendments are editorial in nature and should not be construed to narrow the scope of the claims.

With regard to the objection of claims 1-13, Applicants believe that each ground of objection has been overcome by the wording of the amended claims. Specifically, the terms "many", "a" and "of" have been added to claims 1 and 4 in accordance with the Examiner's

suggestion. Further, claim 9 has been amended to depend on previous claims 1, 4 or 6 as required by the Examiner. Thus, in view of the changes to claims 1, 4 and 9, Applicants believe that the objections to the claims cannot be sustained and should be withdrawn.

With regard to the rejections of claims 1, 9 and 10 under 35 USC § 112, first paragraph, these rejections are deemed to be untenable in view of the amended claims and is thus respectfully traversed.

Claim 1 has been clarified since the subject matters of claims 2, 3, 5, 7 and 8 have been incorporated therein. With respect to claim 9, the mutant is obtained by the method of the present invention. The method of the present invention is not such a conventional method as that introducing a mutation into a specific gene thereby modifying cells or organisms. The claimed method randomly introduces mutations into genomic DNA and selects cells or organisms having tolerance to a given condition (i.e., a stress condition). As strategies for coping with stress might be varied in cells and organisms, a modification of a specific gene should be less effective to obtain a mutant tolerant to the stress condition. The present method makes it possible to obtain various mutants of being more tolerant to the stress condition. Applicants believe that one of skill in the art can also obtain the mutant of claim 9 in accordance with the disclosures. Also, they can identify and obtain the mutated gene by a well-known method in the art.

With regard to the rejection of claims 1-13 under 35 USC § 112, first paragraph, this rejection is deemed to be untenable and is thus respectfully traversed. This rejection has been overcome by the amendment to claim 1. Specifically, the phrase "organism individuals" has been deleted and the claims are now directed to a method for mutagenesis of a gene, which comprises

introducing many more point mutations into one strand of double-stranded genomic DNA of a cell than into another strand.

With regard to the rejections of claims 1-4, 9 and 10 under 35 USC § 102(b) as being anticipated by Fijalkowska et al or Lin et al., these rejections are deemed to be untenable in view of the amended claims and are thus respectfully traversed.

To constitute anticipation of the claimed invention, a single prior art reference must disclose each and every material element of the claim. Here, in this case, both Fijalkowska et al. or Lin et al. fail to teach or suggest the present invention since the subject matter of newly amended claim 1 incorporates the subject matter of claims 5, 7 and 8 which are not taught or suggested by Fijalkowska et al. or Lin et al.

Thus, in view of the amendments to claim 1, these rejections can no longer be sustained and should be withdrawn.

With regard to the rejection of claims 9 and 10 under 35 USC § 102(b) as being anticipated by Pan et al., Garza et al, Herbst et al., Ota et al., or Sussel et al., these rejections have been deemed to be untenable and are thus respectfully traversed.

The Examiner points out that Pan et al. employ “stepwise selection”. However, Pan et al. never discloses nor suggests the special step (b) in claim 1. Pan et al. did not repeat the step of introduction of mutation under a certain condition and the step of selection of mutant under a selection condition without introduction of mutation in the condition that the steps of introduction of mutation the second time and thereafter are carried out under the same selection condition as that in the steps of mutant selection immediately therebefore. Furthermore, the present method

makes it possible to obtain a mutant E. coli able to grow in 10,000  $\mu$  g of ampicillin. The mutant of Pan et al. grow in 100  $\mu$  g of ciprofloxacin. Although the antibiotic is not the same, the tolerance rate of the mutant of this invention is much superior to that of Pan et al. We believe the claimed method and mutant are clearly inventive over Pan et al. as well as the other reference.

Thus, in view of the above, these rejections under 35 USC § 102(b) cannot be sustained and should be withdrawn.

With regard to the rejection of claims 1-6 and 9-12 under 35 USC § 103(a) as being unpatentable over Fijalkowska et al. in view of Lin et al., and further in view of either Imamoto et al. or alternatively, Iwaki et al., this rejection is deemed to be untenable and is thus respectfully traversed.

To establish a prima facie case of obviousness, the cited references in combination must teach or suggest the invention as a whole including all the limitations of the claims. Here, in this case, none of the cited reference teach or suggest the present invention since the unobvious subject matter of claims 5, 7 and 8 have been incorporated into claim 1.

Thus, in light of the amendment to claim 1, this rejection cannot be sustained and should be withdrawn.

With regard to the rejection of claims 1-13 under 35 USC § 103(a) as being unpatentable over Fijalkowska et al. and Lin et al. in view of either Imamoto et al. or Iwaki et al., and further in view of Pan et al., this rejection is deemed to be untenable for the same reason as note above and is thus respectfully traversed. As stated earlier, Pan et al. never discloses nor suggests the special

step (b) in claim 1. Thus, since the cited references in combination fails to teach or suggest all the limitation of the claims, this rejection cannot be sustained and should be withdrawn.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

In view of the foregoing amendments and remarks, it is respectfully submitted that the Application is now in condition for allowance. Such action is thus respectfully solicited.

If, however, the Examiner has any suggestions for expediting allowance of the application or believes that direct communication with Applicants' attorney will advance the prosecution of this case, the Examiner is invited to contact the undersigned at the telephone number below.

Respectfully submitted,

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## VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows:

1. (Amended) A method for mutagenesis of a gene, which comprises:

(a) introducing [much] many more point mutations into one strand of double-stranded genomic DNA of a cell [organism individual] than into another strand, wherein the point mutations are randomly introduced into four kinds of bases, and wherein the cell is derived from a mutant cell strain having a mutator gene which causes a defect of mutation repair mechanism under a certain condition, and

(b) repeating a step of introduction of mutation into genomic DNA under the certain condition and a step of selection of mutant under a selection condition without introduction of mutation, wherein the steps of introduction of mutation at the second time and thereafter are carried out under the same selection condition as that in the steps of mutant selection immediately therebefore.

4. (Amended) The method of mutation according to claim [3] 1, wherein the mutator gene is one or more mutator genes selected from a group consisting of dnaQ, dnaE, mutL, mutS, mutH, uvrD and dam.

6. (Amended) The method according to claim [5] 1, wherein the condition for the defect of the mutation repair mechanism is a certain temperature.

9. (Twice Amended) A mutant of a cell, [or organism individual where] wherein mutation is introduced into the genomic DNA of the cell by any one of the methods of claims 1, 4 or 6 [to 8

or 11-13].

10. (Amended) A mutated gene introduced into and [which is] isolated from the mutant of claim 9.

## Abstract

This application provides a method for mutagenesis of a gene, which comprises introducing much more point mutations into one strand of double-stranded genomic DNA of cell or organism individual than into another strand. In accordance with such a method, it is now possible to efficiently and effectively construct various useful mutants of microorganisms, cells or organism individuals. It is also now possible by analyzing the mutating conditions of the gene to clarify the mechanism of drug resistance, to estimate the occurrence of a novel insensible microorganism or to develop a drug therefor, to analyze the mutation of an oncogene and the mechanisms of cancer metastasis and increase in malignancy, to develop a therapeutic method using these mechanisms, etc.